

Sentinel lymph node in vulvar cancer – a pilot study to identify and assess the diagnostic value

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Introduction. Vulvar cancer is the fourth most common gynaecological malignancy, the treatment results of which still remain unsatisfactory. The poor outcome is probably due to advanced stages of the disease at diagnosis and/or from delays in the onset of complementary therapy which is necessary in case of the presence of lymph node metastases. These delays are, in turn, usually caused by complications brought on by extensive surgical excision.

Aim. Our aim was to conduct a pilot study in order to design an optimal method of sentinel lymph node identification in patients with squamous cell carcinoma of the vulva and to assess the diagnostic value of the sentinel lymph node biopsy in this particular malignancy.

Material and methods. The patients were considered as eligible for the study if they were to undergo radical vulvectomy due to squamous cell carcinoma stage T1-2, N0-2, M0. The sentinel lymph node was identified intraoperatively using a combined method: lymphoscintigraphy (gamma-camera scanning after the administration of Technetium Tc99m) and Patentblau V staining.

Results. All the specimens were referred for histopathological examination as the lymph nodes were found to contain lymphatic tissue. The positive prognostic value of simultaneous application of both methods of sentinel lymph node identification was found to be 94.8%. Lymph node metastases were found in 13 cases out of a total of 40 operated inguinal basins. In 3 cases metastases were found in the sentinel nodes, while the remaining unilateral lymphatic nodes were cancer-free. We found no case of inguinal lymph node metastases in which the sentinel lymph node would be free of cancer metastases.

Conclusions. It may be assumed that the combination of lymphoscintigraphy and Patentblau dyeing is an effective method of sentinel lymph node identification. In order to assess the diagnostic value of sentinel lymph node biopsy in the treatment of squamous cell carcinoma of the vulva it is necessary to study a larger group of patients over a longer follow-up period.

Identyfikacja i ocena wartości diagnostycznej węzła wartowniczego w raku płaskonabłonkowym sromu w oparciu o doświadczenia własne

Wstęp. Rak sromu jest IV co do częstości występowania lokalizacją nowotworu złośliwego kobiecych narządów płciowych. Wyniki leczenia są niezadawalające. Powodem niepowodzeń terapii mogą być: często wysokie zaawansowanie choroby lub opóźnienie rozpoczęcia leczenia uzupełniającego u chorych z przerzutami do węzłów chłonnych. Ten fakt jest zazwyczaj spowodowany powikłaniami, będącymi rezultatem rozległego zabiegu operacyjnego.

Cel. Celem pracy jest przeprowadzenie badania pilotażowego dla opracowania optymalnego sposobu identyfikacji i oceny wartości diagnostycznej węzłów wartowniczych w grupie chorych na raka płaskonabłonkowego sromu.

Materiał i metoda. Do badania zakwalifikowano chore na raka płaskonabłonkowego sromu w stopniach zaawansowania T1-2, N02, M0, u których wykonywano radykalne usunięcie sromu. Węzeł wartowniczy identyfikowano śródoperacyjnie przy pomocy gamma kamery po wcześniejszym podaniu izotopu technet 99^m oraz na podstawie ich wybarwienia po podaniu barwnika Patentblau V.

Wyniki. We wszystkich tkankach przesłanych do badania histopatologicznego jako węzły chłonne odnaleziono utkanie chłonne. Pozytywna wartość prognostyczna stosowania obu metod identyfikacji węzła wartowniczego wynosi 94.8%. Przerzuty do węzłów chłonnych stwierdzono w 13 na 40 operowanych pachwin. U 3 chorych stwierdzono przerzuty w węzłach wartowni-

czych, natomiast nie stwierdzono ich w pozostałych węzłach chłonnych. Nie stwierdzono przypadku znalezienia przerzutów w węzłach pachwinowych i jednocześnie nieobecności przerzutów w węzłach wartowniczych.

Wniośki. Wydaje się, że metoda limfoscintygrafii i barwienia węzłów barwnikiem Patentblau jest skuteczna w identyfikacji węzła wartowniczego. W celu oceny wartości diagnostycznej węzła wartowniczego konieczne jest przeprowadzenie badania na większej liczbie chorych i dłuższa obserwacja chorych po leczeniu.

Key words: vulvar cancer, sentinel node, lymphoscintigraphy

Słowa kluczowe: rak sromu, węzeł wartowniczy, limfoscintygrafia

Introduction

In Poland about 200 patients each year die of vulvar cancer [1]. The majority of these deaths are caused by advanced cancer (stages III and IV, i.e. with the presence of lymph node metastases) [2]. What is more, treatment results are poor. This particular malignancy usually affects women over 60 years of age [3], a majority of whom are seriously compromised by other diseases, such as coronary heart disease, arterial hypertension and diabetes. The present standard in the treatment of vulvar cancer at our institution is to perform a radical surgical excision of the vulva with bilateral inguinal lymphadenectomy. The actual stage of the disease, which should dictate the choice of treatment protocol, is in fact assessed during postoperative histopathological evaluation of the surgical specimen. If routine hematoxylin/eosin staining of the tissue samples reveals metastases to the inguinal lymph nodes the patient is referred for radiotherapy of the pelvis encompassing the inguinal lymph-node fields. However, surgical treatment consists of a wide excision performed in a microbiologically comprised field (the vulva being a potentially infected area) and often leads to septic complications. These, in turn, delay recovery and, especially in patients of an advanced age and in poor biological condition with a number of co-existent diseases, significantly increase postoperative mortality. On average the patients remain in hospital for a period between 2 and 6 weeks. This delays the onset of adjuvant treatment – which is in fact a crucial element of the entire therapy when lymph node metastases are present – seriously affecting the patients' five-year survival results. On the other hand, lymphadenectomy, when performed unnecessarily in patients with no lymph node metastases, distinctly affects the efficacy of the immune system thus, causing repercussions which are difficult to foresee. In view of all this it is very important to assess the actual stage of vulvar cancer with the aid of the least invasive methods. Such evaluation will, on the one hand, save a number of patients from the handicap of unnecessary lymphadenectomy and, on the other, will speed up the onset of adjuvant radiation therapy.

Study aim

The aim of our study was to design and introduce an optimal method of sentinel lymph node identification in patients with stage I and II squamous cell carcinoma of the vulva and to assess the diagnostic value of sentinel lymph node biopsy in this particular malignancy.

Material and methods

The material consisted of consecutive patients treated at the Department of Gynaecological Oncology of the Maria Skłodowska-Curie Memorial Cancer Center in Warsaw (MSCMCC) between January 1st 2001 and April 30th 2002 who fulfilled the following criteria:

- WHO (Zubrod) overall performance status I-II.
- Histopathologically confirmed invasive squamous cell carcinoma of the vulva.
- Clinical advancement stage T1-2, N0-2, M0.
- No history of previous treatment for either vulvar cancer nor any other malignancy.
- Informed consent submitted in writing.

If all the above criteria were fulfilled the patients would undergo thorough medical examination and a number of additional diagnostic investigations including chest X-ray and abdominal ultrasound (which are a part of routine before oncological surgery at the MSCMCC) before final qualification.

All patients enrolled into the study were administered Tc99m isotope of Technetium (activity 1.2 mCi) in the direct vicinity of the vulvar tumour 24 hrs. before surgery. One hour after isotope administration we performed a scintigraphic examination of the inguinal lymph nodes in order to assess the areas of isotope accumulation. Scintigraphy was performed with a double-headed high-resolve VARICAM gamma-camera (manufactured by ELSCINT), with a low-energy collimator. The data was registered on a 256 x 256 matrix, zoom 1, over a time of 10 minutes (aprox. 100 000 counts from each head). The injection site was covered with leaden shielding (0.5 cm thick). EXPERT software, by ELSCINT, was used for the processing of results and figures.

All patients were operated by a team of specialists from the Department of Gynecological Oncology of the MSCMCC. In order to visualise the sentinel lymph node a 2.5% solution of Patentblau V was injected transcutaneously in the direct vicinity of the vulvar tumour 10 minutes before skin excision. Inguinal lymph node excision was performed as the first step of the procedure. The sentinel nodes were identified with a hand-held gamma-camera equipped with a radiation meter (Navigator GPS – Gamma Positioning System) in the following sequence of measurements: (1) above the skin of the inguinal area, (2) after skin incision identifying the sentinel nodes “*in vivo*”. After the removal of the sentinels (either one or multiple nodes over which we detected the highest isotope accumulation) the radioactivity of the wound itself was measured and noted as the so-called “background uptake”. If no isotope accumulation was detected within the entire inguinal node area the nodes which were stained with Patentblau V dye were removed and referred for histopathological examination as the sentinels. Surgery was continued with radical excision of the entire group of inguinal lymph nodes and the vulva, in accordance with the present MSCMCC standards of treatment of T1-2, N 0-2, M 0 squamous carcinoma of the vulva.

All tissue specimens underwent histopathological evaluation with routine hematoxylin/eosin staining and immunohistochemical evaluation aimed at discerning the presence of cytokeratines within the cancer cells. The sentinel lymph nodes were examined independently from the remaining lymph nodes.

Results

Between January 1st 2001 and April 30th 2002 36 women were treated for vulvar carcinoma at the Department of Gynaecological Oncology of the MSCMCC in Warsaw. Of these 36 patients 20 fulfilled the criteria necessary to enter the study. Data analysis has allowed us to come up with the following results:

1. Gamma-camera examination and staining with Patentblau V dye both allow to localise lymph nodes, because all the specimens identified as such were proven to contain lymphatic tissue
2. Gamma-camera examination has allowed for sentinel lymph node identification in 36 cases (90%) of a total of 40 excised inguinal basins. The positive prognostic value of this method was 93.7%, while its negative prognostic value was 94.1%. The sensitivity and specificity of this method set at 97.9% and 88.2%, respectively.
3. Intraoperative evaluation established 34 cases of Patentblau V-stained lymph nodes (85%) in a total of 40 excised inguinal basins. The positive prognostic value of Patentblau V-staining was 96%, while its negative prognostic value was 96.7%. The sensitivity and specificity of this method set at 86.9% and 78.6%, respectively.
4. The positive prognostic value of both the methods combined was 94.8%, while their negative prognostic value was 95.4%. Their combined sensitivity and specificity set at 92.4% and 83.4%, respectively.
5. Among the 20 studied patients inguinal lymph node metastases were found in 11 cases; in 9 patients they were bilateral, in the remaining 2 patients they were limited to one side.
6. In 10 (25%) of the 40 excised inguinal basins metastases were found both in the sentinel lymphnodes and within the other unilateral nodes.
7. In 3 cases (7.5%) metastases were found in the sentinel lymph nodes, while the remaining unilateral inguinal nodes were metastases-free.
8. In no case of metastatic cancer found within the inguinal nodes were the sentinel lymph nodes free of metastases i.e. if we found metastatic cancer within the nodes of one inguinal basin, then metastases were always present also in the sentinel nodes.
9. No complications or side-effects of the sentinel lymph node identification procedures were observed.

Discussion

The sentinel lymph node is defined as the first lymph node reached by the lymph flowing in from the drainage area in which the tumour is located. In 1992 Morton reported a technique for sentinel lymph node identification in patients with melanoma of the skin [4]. Since then numerous studies had been conducted in order to assess whether the results of histopathological examination of the sentinel lymph node are representative in comparison to the state of other lymph nodes of the same basin. The

applicability of this method in malignancies other than melanoma of the skin, for example in breast cancer [5] was another widely studied issue. There is also extensive research aimed at assessing the diagnostic value of sentinel lymph node biopsy in patients treated for vulvar cancer. One goal is to develop an optimal method of sentinel lymph node identification. The other is to evaluate whether sentinel lymph node biopsy may be considered representative in cases of vulvar carcinoma.

As far as sentinel lymph node identification is concerned two procedures are being recommended – one is staining with injected dye – for example with Patentblau V. The main assets of this method are: relatively low cost (one 2 ml vial is an adequate dose for one patient), safety (there are no reports of life-threatening side-effects) and simplicity. The drawback of lymph node staining is that it requires very precise timing – i.e. exact synchronisation between administering the dye intracutaneously in the direct vicinity of the tumour and visualising the sentinel lymph node during surgery exactly 10 minutes after the injection. If intraoperative visualisation is in any way delayed the dye will reach further structures of the lymphatic system and render sentinel lymph node identification not only difficult, but sometimes even impossible. Levenback maintains that this method is cost-effective and specific, while its sensitivity increases with acquired practice [6, 7, 8]. The other sentinel lymph node identification technique is that of measuring the radioactivity of Technetium Tc99m isotope over the lymph nodes. Lymphoscintigraphy is a diagnostic tool which has been known for years, but which was to a certain extent forsaken due to the development of computerised tomography, magnetic resonance imaging and ultrasonography. The last few years, however, have seen its rebirth, brought on by the appearance of the gamma-camera, which allows to measure the radioactivity of the isotope directly over the lymphatic nodes. The isotopes which are currently in use carry no risk for the patient, but their injection is rather painful. In cases of vulvar cancer there were some minor changes in the procedure. It had been presumed that the isotope should be injected into the tumour, but it has been proven that its administration in the direct vicinity of the tumour is just as effective. A significant limitation of this method is obtaining the gamma-camera itself, the value of which on the Polish market is estimated at 80 000-100 000 PLN. Nevertheless, the combination of both these methods allows to increase the sensitivity of sentinel lymph node identification. This aspect has been stressed by many authors [9, 10, 11] and is also a finding of our pilot study. Within the inguinal basins, which we have examined, the lack of sentinel node staining was observed more often (6 cases) than the lack of discernible isotope activity (4 cases). At the same time in two cases the lymph node identified as the sentinel was identified by staining only, as no radioactivity was discerned over it. In these two particular cases both the sentinel lymph nodes and the other nodes of the inguinal basin were free of metastases. It must be stressed, that the ability to identify the sentinel node is only one aspect of this issue.

A crucial query, which is still to be resolved, is the diagnostic value of the sentinel lymph node biopsy. There is a lot of data to prove that in cases where no metastases are found within the sentinel lymph node the entire inguinal basin may be considered free of vulvar cancer metastases [12, 13, 14]. The pilot study which we have performed supports these findings. In all those cases where the lymph node identified as the sentinel was free of metastases so where the remaining nodes removed in the course of inguinal lymphadenectomy. In view of such findings one may hope that sentinel lymph node biopsy will, in the near future, allow to limit the extension of lymph node excision in the surgical treatment of patients with vulvar cancer. Numerous literature reports have shown that early adjuvant chemo-radiotherapy plays a crucial part in increasing five-year survivals of patients treated for vulvar cancer. On the other hand, surgical wounds after extensive inguinal lymphadenectomies are difficult to heal. The hospitalisation period often extends to six weeks. Even if one ignores the sheer economical aspects of such long hospital stays, there remains the most important issue – the delay in the onset of adjuvant treatment, which puts both the patients' health and their lives at stake. At the same time, lymphadenectomies performed unnecessarily, in patients with no metastases, do nothing to increase the efficacy of oncological treatment. In fact they only increase the patients' handicap. The issue is not only that of unnecessary human suffering brought on by needless surgery – there are also other consequences to consider. After inguinal lymphadenectomy numerous complications are observed – oedema of the lower extremities, limited movement and different kinds of pain, including paraesthesia. It must also be stressed that we do not know the consequences of destroying an entire level of the lymphatic system on the immunological competence of the human organism. The mean number of lymph nodes which we have removed in the course of inguinal lymphadenectomies was 6 in the right basin and 5 in the left basin (sentinels and unilateral lymph nodes). The largest number of excised nodes was 14, the smallest – 2 (one sentinel and one “ordinary” inguinal lymph node). This lowest number of excised nodes was

observed only in 2 cases of a total of 40 inguinal lymphadenectomies. In one of these two particular cases the sentinel node contained metastases, while the other node was cancer-free. In the other case both the nodes were free of cancer metastases. Due to the use of the hand-held Neoprobe gamma-camera the site of the nodes could be determined with great precision, which allowed to perform a small skin incision and, thus, a much smaller wound. In the course of this novel approach we also observed improved healing of the inguinal wounds, thus shortening the hospital stay. The mean duration of postoperative hospitalisation was 16.7 days, which is shorter than the hospital stays reported by other authors (up to 6 weeks) [2].

Through limiting the extent of surgical excision it was possible to reduce the frequency of typical complications to a significant extent. Under such circumstances adjuvant treatment could be introduced much sooner and, in due course, greatly improve the five-year survival ratio.

Conclusions

Lymphoscintigraphy and Patentblau V staining have been proven to be an efficient tool in the localisation of sentinel lymph nodes in patients with vulvar cancer. No false negative results of sentinel lymph node biopsies were observed. Conclusions affecting the routine tactics of vulvar cancer treatment can only be made after an analysis of a larger study group. Moreover, both local recurrence and/or the appearance of distant metastases can only be assessed after at least 3 years of follow-up. It has been established that in this particular group of patients recurrence is usually observed during the first 2 years after radical treatment completion. At the same time one cannot avoid raising questions as to the nature of the mechanisms behind this particular malignancy, especially in view of the fact that recurrences are observed in cases of initial early advancement and after radical treatment. Determining possible correlation between the frequency of local recurrence or appearance of distant metastases and the presence of micrometastases within

Table I. Evaluation of the sensitivity and specificity of Patentblau V staining and Tc99m lymphoscintigraphy for sentinel lymph node identification

Identification method	No. of positive localisations [%]	Positive prognostic value	Negative prognostic value	Sensitivity	Specificity
Staining	34/40 [85%]	96%	96.7%	86.9%	78.6%
Gamma-camera	36/40 [90%]	93.7%	94.1%	97.9%	88.2%
Staining & Gamma-camera	–	94.8%	95.4%	92.4%	83.4%

both the sentinels and other lymph nodes, might help to answer these important questions. It could also help to reassess the true value of the present-day treatment regimes.

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